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THE EFFECTS OF INTERMITTENT HIGH ASBESTOS EXPOSURE (PEAK DOSE LEVELS) ON THE LUNGS OF RATS

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Summary.—Four groups of rats were treated by inhalation with the UICC preparations of amosite or chrysotile in order to explore the effects of intermittent high dust concentrations (peak dosing). For each of the 2 asbestos types one group of rats was treated for 5 days each week, 7 h a day, for 1 year. Two other groups were treated with amosite or chrysotile at 5 times the previous dose for 1 day each week for 1 year. Results showed that the lung dust levels of both chrysotile or amosite in the lungs of rats after the 12-month inhalation period were similar regardless of whether "peak" or "even" dosing had been used. During the following 6 months, asbestos was cleared from the "peak" chrysotile group more slowly than the "even" chrysotile group but clearance from the "peak" amosite group was faster than that found after "even" dosing with amosite. Levels of early peribronchial fibrosis were generally lower for the "peak" dosing groups than for "even" dosing although levels of interstitial fibrosis were slightly higher following "peak" dosing. The incidence of pulmonary neoplasms did not differ between the "peak"-dosing and "even"-dosing experiments. These findings therefore give no indication that short periods of high dust exposure in an asbestos factory would result in a significantly greater hazard than would be indicated by the raised overall dust counts for the day in question.

INHALATION STUDIES published by a number of authors have shown that the laboratory rat is a suitable model to use in the study of asbestos-related lung disease. In humans asbestos exposure is known to result in the development of pulmonary interstitial fibrosis, bronchial carcinoma and pleural or peritoneal mesothelioma, and similar conditions can be produced experimentally in rats. Gross and de Treville (1967), Wagner *et al.* (1974), Reeves, Puro and Smith (1974) and Davis *et al.* (1978) have reported interstitial fibrosis and bronchial carcinomas following experimental inhalation of several asbestos types. Wagner *et al.* (1974) reported the occurrence of occasional pleural mesotheliomas following asbestos inhalation and Davis *et al.* (1978) also found one peritoneal mesothelioma.

However, all these studies used a constant even dose of the various asbestos types and this is not closely comparable to the situation encountered by workers in modern asbestos mills and factories. In these workplaces the overall asbestos exposures are generally very low but occasional breakdowns in the ventilation to any piece of machinery or even periods of maintenance work on machinery can produce high localized dust levels for short periods of time. It has been suggested that these short "peak" doses, which hardly change the overall daily dust counts for the area or factory concerned, may be dangerous because the pulmonary clearance mechanism of exposed workers may become saturated (Holmes, 1972). This could result in a level of asbestos retention far greater than would occur if the same dose was spread over a long period. It was considered important to test this hypothesis under experimental conditions and the present paper reports the findings from experiments using the UICC samples of amosite and chrysotile.

MATERIALS AND METHODS

For previous inhalation studies on the effects of asbestos dust, it has been the practice in our laboratory to expose animals to a constant dust dose for 7 h each day, 5 days each week, for a total period of 12 months, an experimental regime hereafter referred to as "even" dosing. In order to obtain information on the effects of "peak" dosing, however, it was decided to expose 2 groups of animals to 5 times the usual dose for 1 day each week. Previous studies (Davis *et al.*, 1978) had used constant doses of UICC amosite at 10 mg/m^3 and chrysotile at both 10 mg/m^3 and 2 mg/m^3 of respirable dust. It would have been desirable, therefore, to study "peak" exposures using dust levels of amosite and chrysotile at 50 mg/m^3. This was possible with amosite but not with chrysotile, since it was found that at high densities the fibres flocculated within the inhalation chamber and it was not feasible to obtain levels of respirable dust much above 20 mg/m^3. It was decided, therefore, to compare the effects of a cloud of chrysotile at 10 mg/m^3 administered for 1 day each week with previously obtained data on the effects of "even" dosing with this material at 2 mg/m^3. The "peak" amosite studies utilized a 50 mg/m^3 cloud and the results were compared to "even" dosing at 10 mg/m^3.

The dust clouds were generated using a modified Timbrell dust generator (Timbrell *et al.*, 1970) and the inhalation chambers were of similar design to Timbrell's. The dust was size selected by a cyclone system (Beckett, 1975) before being added to the chamber air stream. This ensured a higher proportion of respirable dust in the clouds. Gravimetric monitoring was carried out during dusting and the daily mass concentration measurements were obtained for all the chambers. The NCB MRE sampler (Cassella Type 113A—Dunmore, Hamilton and Smith, 1964) was used to measure the concentrations in the amosite chambers. At 10 mg/m^3 with chrysotile, this instrument had been found to undersample and a vertical elutriation system (Beckett, 1975) was therefore used to monitor the chrysotile clouds.

Additional dust samples were obtained for the estimation of fibre dimensions using the standard sampling method described by the Asbestosis Research Council (1971). Each membrane filter sample was taken using an open Gelman filter holder facing downwards at a flow rate and sampling time calculated to give an optimum density for the microscopical examination (1–3 fibres per graticule area). Fibre length and diameter distributions were obtained partly by phase-contrast microscopy and partly by scanning electron microscopy (Beckett, 1973), using a Cambridge Instruments S600 scanning electron microscope.

Studies on the effects of "peak" dosing of asbestos utilized groups of 48 male random-bred white SPF Wistar rats of the AF/HAN strain. These were exposed for a period of 12 months and groups of 4 animals were killed at 12 and 18 months after the start of dusting for the estimation of pulmonary fibrosis as well as dust deposition and retention. The experiment was terminated at 29 months for comparison with previous studies from this unit (Davis *et al.*, 1978) and lungs from 6 animals in each group were used for estimations of advanced fibrosis. Lungs from the remaining animals were examined only for the presence of pulmonary neoplasms. Tissue used for histological examination was fixed with 10% formol saline solution and embedded in paraffin wax: lungs were fixed by inflation *in situ* until they filled the thoracic cavity. Sections were stained with either haemotoxylin and eosin, van Giesen's method for collagen or Gordon and Sweet's stain for reticulin. Levels of pulmonary fibrosis were calculated using the methods previously described by Davis *et al.* in 1978. Both lungs and heart were embedded together and sections were cut in the coronal plain to include parts of all lobes. Sections were cut at 4 different levels in each block and were at least 1 mm apart, and groups of serial sections were mounted from each of these levels for use with the different staining techniques. For all lesions the H. and E. sections were scanned with the light microscope using an eyepiece graticule consisting of a 1cm square subdivided into 100 units of 1 mm^2. Viewing magnification was $\times 60$. The area of regions of interstitial fibrosis was estimated for each slide by counting the number of grid squares involved and presenting the results as a percentage of total lung area in the section. An average figure for the animal was produced by combining the result from all 4 sections. The early peribronchial fibrotic lesions were usually much smaller than one grid square at the magnification involved and since they were associated with the respiratory bronchioles they were also widely scattered. For this type of small lesion, the calculations were based on the number of squares that contained the small areas of fibrous tissue and the results from all four sections were again presented as a percentage.

Asbestos retained in the lungs of selected animals was recovered by a low-temperature ashing process. This was conducted in a stream of oxygen excited by a radio frequency discharge (Gleit and Holland, 1962). Any residual lung salts were removed by washing the samples in 3 ml of cold (20°) 0·2M HCl before gravimetric estimations of the amounts of asbestos recovered were made using the infra-red spectrophotometric techniques described by Middleton, Beckett and Davis (1977). The amounts of dust retained in the lungs of rats killed 12 and 18

months after the start of dusting was estimated from ashed residues of the left lung in each case, the right lung being retained for histological examination. Studies in this laboratory have shown that the asbestos content ratio between left and right lungs following experimental asbestos inhalation in rats is 0·6:1 and this correction factor was therefore used to calculate the total pulmonary dust burden of each animal.

RESULTS

The results of "peak" dosing studies using amosite and chrysotile have been compared throughout with the results of experiments where the same dust types were administered to rats at the "even" dose levels. The dust parameters for the 4 chambers during the dusting period are given in Table I. The mass concentrations obtained were all very close to the target levels. During the study, a series of dust samples was obtained on Nuclepore filters and these were utilized for measuring size distribution of the fibres using a scanning electron microscope. The fibre length and diameter distributions from the peak dusting chambers appeared identical to those obtained from the same dust types administered at "even" dose levels (Figs 1 and 2). The chrysotile cloud had a higher proportion of fibres over 10μm in length than the amosite and only the chrysotile cloud had any significant proportion of fibres as long as 50 μm.

The survival times for the animals for the 4 inhalation chambers are shown in Table II. These indicate that there were no overall differences in longevity between animals treated with different asbestos clouds. Histological examination of lung tissue from animals in the peak dusting studies showed types of dust lesions identical to those present in "even"-dosed animals. The earliest lesions consisted of deposits of dust and granulation tissue around the respiratory bronchioles and alveolar ducts. In animals treated with chrysotile the granulation tissue contained many giant cells as well as macrophages and fibroblasts but giant cells were rare

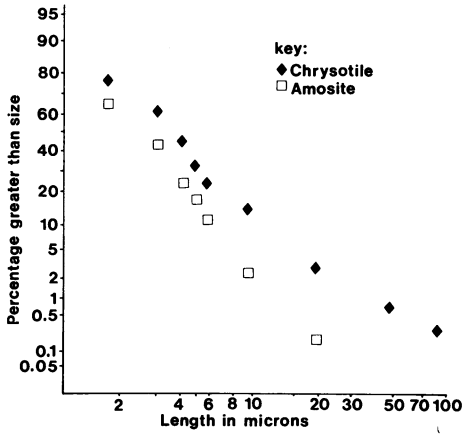

FIG. 1.—Length distributions of fibres longer than 0·6 μm for the chrysotile and amosite clouds used in the study of high intermittent doses. (Scanning electron microscope measurements.)

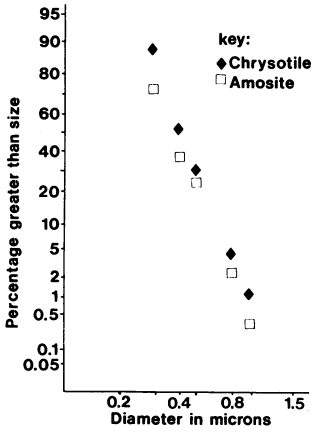

FIG. 2.—Diameter distribution of fibres broader than 0·2 μm for the chrysotile and amosite clouds used in the present study on high intermittent doses. (Scanning electron microscope measurements.)

TABLE I.—*The mean mass of the 4 UICC asbestos clouds over the exposure period*

	Chrysotile (even dosing)	Chrysotile (peak dosing)	Amosite (even dosing)	Amosite (peak dosing)
Target concentration (mg/m^3)	2·0	10·0	10·0	50·0
Mean concentration achieved (mg/m^3)	2·0	9·0	10·0	49·2
Mean ratio of total to respirable dust	1·3 : 1	1·1 : 1	1·15 : 1	1·13 : 1

TABLE II.—*Number of animals surviving in the different inhalation groups throughout the study. The experiment was terminated at 29 months. Groups of 4 animals from each chamber were killed at both 12 and 18 months*

Dust cloud	Months after start of exposure			
	12	18	24	29
Chrysotile 10 mg/m³ (peak dose)	48	43	29	7
Chrysotile 2 mg/m³ (even dose)	48	40	26	7
Amosite 50 mg/m³ (peak dose)	48	44	31	8
Amosite 10 mg/m³ (even dose)	47	40	22	11

in animals treated with amosite. These early lesions contained considerable amounts of reticulin but only small amounts of collagen were found at this stage. In some cases the normal epithelial lining of the respiratory bronchioles, alveolar ducts and associated alveoli became replaced by rounded epithelial cells of bronchiolar type. It was not, however, possible to determine whether or not this was due to hyperplasia of the bronchiolar lining or metaplasia of the alveolar epithelial cells. This change was usually found associated with areas of peribronchiolar granulation tissue and fibrosis but it could occur on its own. More advanced changes consisted of the thickening of alveolar septa over quite large areas of lung tissue (Fig. 3). In these areas of interstitial change the alveoli were lined with rounded cells, probably Type 2 pneumocytes, and there was an increase in the thickness of the reticulin network in the septal walls. No collagen was present in the early stages but in the oldest animals some areas of interstitial fibrosis showed very marked thickening of the alveolar septa which stained strongly positive for collagen.

In some areas, however, an alternative to advanced fibrosis was the continued growth of the rounded alveolar epithelial cells with subsequent compression of the alveoli to produce some adenomatous appearance. In some cases definite adenomas had formed in these areas. In a few animals small areas of squamous metaplasia of the alveolar epithelium were also found.

Quantitative estimations of these lesions in both animals subjected to "peak" doses and those which had received the same amount of asbestos at an "even" rate are given in Table III. From these data it can be seen that for chrysotile the levels of early peribronchiolar fibrosis were significantly lower following peak dosing than even dosing ($P < 0{\cdot}05$). With amosite, however, there were no significant differences between the 2 dusting regimes. Similarly there appeared to be no progression of the small lesions of peribronchial fibrosis after the end of the dusting period with either method of dosing. In fact the oldest animals of each group showed less peribronchiolar fibrosis than those examined at either 12 or 18 months after the start of dusting. However, this might be due to an increase in areas of interstitial fibrosis which enclosed and masked earlier areas of fibrosis close to the bronchioles. The extension of bronchial epithelium to alveolar ducts and alveoli did not differ significantly between peak and even dosing for either amosite or chrysotile. Levels of pulmonary interstitial fibrosis were similar for the peak and even dose groups of both asbestos types at both 12 and 18 months from the start of dusting. At 29 months, however, animals from both the peak dosing experiments appeared to have more pulmonary interstitial fibrosis than those treated with the same type of asbestos at "even" dose levels although these differences were not statistically significant.

The incidence of neoplasms of the lung and mesotheliomas found in the 4 experimental groups is shown in Table IV. The figures for the peak and even-dose studies show no significant differences. The peak amosite study, however, did produce 2 bronchial carcinomas where none had occurred with even dosing of the same material. The tumour incidence from sites

FIG. 3.—An area of pulmonary interstitial fibrosis in the lung of a 32-month-old rat after the inhalation of chrysotile asbestos. × 200.

other than lung and excluding mesotheliomas is shown in Table V and it can be seen that there were no significant differences between the 4 groups of animals under study.

The weights of asbestos extracted from the lungs of animals in the different inhalation groups are summarized in Table VI. This shows that the amount of chrysotile present at the end of the 12-month dusting period is extremely close regardless of whether the inhalation dose was administered evenly during the week or concentrated into a single day. In animals treated with amosite, however, the retention of inhaled dust at the end of the dusting period was higher for "peak" dosing than for "even". Long-term clearance of chrysotile from the rat lungs appeared to be slower following "peak" dosing, while clearance of amosite appeared faster in the "peak" dosing experiment. In all the cases, however, statistical examination of the figures showed no significant differences.

DISCUSSION

The figures for the deposition and retention of asbestos dust reported in the present study appear to refute the idea that short periods of very high exposure to asbestos result in a much higher lung burden of retained dust than might be expected from the greater density of the "peak" dust clouds. The amounts of dust found in the lungs of chrysotile-treated animals at the end of the dusting period for both "peak" and "even" dosing were extremely close. For amosite, animals in the "peak" dosing group did appear to retain more dust than those subjected to "even" dosing, but with only 4 animals in each group the figures were not significantly different. There were no significant differences between the long-term clearance of chrysotile and amosite regardless of whether dust had been administered by either peak or even dosing. These figures suggest that short periods of high dust exposure in asbestos factories are unlikely to result in a much greater lung dust bur-

TABLE III.—*Mean levels and ranges of lung fibrosis produced in rats by clouds of UICC chrysotile and amosite administered at different dose rates (parameters as described in Methods section)*

	Chrysotile 2 mg. Even dose			Chrysotile 10 mg. Peak dose			Amosite 10 mg. Even dose			Amosite 50 mg. Peak dose		
Time after start of exposure (months)	12	18	29	12	18	29	12	18	29	12	18	29
Peribronchiolar fibrosis	10·7 (7·8–12·7)	9·9 (7·5–11·77)	7·53 (5·2–9·0)	6·8 (4·5–8·9)	6·1 (3·7–8·8)	3·9 (2·5–6·5)	4·12 (3·0–5·5)	5·1 (3·8–5·9)	4·2 (2·5–5·5)	6·2 (4·6–7·4)	5·4 (3·2–7·5)	2·9 (1·6–3·8)
Extension of bronchial epithelium to alveolar ducts and alveoli	1·7 (1·1–2·5)	4·03 (2·8–6·6)	1·05 (0·5–1·5)	2·3 (1·2–3·4)	2·6 (1·3–4·0)	1·5 (0·6–2·3)	2·27 (1·6–3·2)	3·9 (1·0–6·0)	3·05 (1·8–5·5)	1·8 (1·3–2·5)	2·1 (1·6–3·3)	1·6 (1·3–2·0)
Interstitial fibrosis	0·35 (0–1·2)	0·83 (0–2·9)	3·86 (0–7·2)	1·3 (0–5·2)	1·05 (0–4·21)	6·8 (0·89–19·5)	0·87 (0–3·24)	0·12 (0–0·4)	2·58 (1·1–5·1)	0·0	1·18 (0–4·6)	5·8 (1·0–11·4)
No. of rats in sample	4	4	6	4	4	6	4	4	6	4	4	6

TABLE IV.—*Pulmonary tumours found in the lungs of rats following either "even" dosing or "peak" dosing at equivalent levels of UICC chrysotile and amosite*

Type of tumour	Chrysotile 2 mg/m³ even dosing 40 animals	Chrysotile 10 mg/m³ peak dosing 43 animals	Amosite 10 mg/m³ even dosing 40 animals	Amosite 50 mg/m³ peak dosing 44 animals
Adenoma	6	4	2	4
Adenocarcinoma	1	1	0	2
Squamous carcinoma	1	1	0	0
Mesotheliomas	1	0	0	0

den than would be indicated by the overall dust counts for the day in question and are in agreement with previously reported experimental work. Thus a series of short-term inhalation experiments (Middleton *et al.*, 1977, 1979) and a long-term inhalation study (Davis *et al.*, 1978) both indicated that, in proportion to the density of the dust cloud, there was no large increase in asbestos dust deposition for exposure levels between 1 mg/m³ and 10 mg/m³. In both long- and short-term studies, the lung dust burden of amphibole dusts was found to be proportionally slightly higher for high doses than for low but for chrysotile the amount of dust in lungs at the end of the dusting period was actually considerably reduced with increasing dust cloud density. In the long-term studies, clearance during the 6 months following the cessation of dusting was slightly slower for high chrysotile doses than for low, but clearance following high doses of amphibole was actually faster. In the short-term studies it was found that the rate of clearance was independent of the type of asbestos or the dust dose.

TABLE VI.—*Calculated weights of asbestos recovered from lung tissue at 7 and 182 days after the end of the dusting period*

Asbestos cloud	No. of animals	Days after exposure	Mean lung dust content (μg)	Percentage clearance between 7 & 182 days
Chrysotile 2 mg/m³	4	7	526	66%
	4	182	180	
Chrysotile 10 mg/m³ (peak dose)	4	7	501	47%
	4	182	270	
Amosite 10 mg/m³	4	7	9169	24%
	4	182	7020	
Amosite 50 mg/m³ (peak dose)	4	7	11226	35%
	4	182	7331	

For methods of calculation see text.

TABLE V.—*Sites of tumours other than lung*

Site of tumour	Chrysotile 2 mg/m³ (even dosing) 40 animals		Chrysotile 10 mg/m³ (peak dosing) 43 animals		Amosite 10 mg/m³ (even dosing) 40 animals		Amosite 50 mg/m³ (peak dosing) 44 animals	
	B	M	B	M	B	M	B	M
Subcutaneous connective-tissue tumours		1			2	3	2	2
Peritoneal connective-tissue tumours		1		1	1	1		
Osteosarcomas		1						
Testicular tumours	1				4		2	
Squamous tumours of the epidermis				2	1			
Parotid tumours								2
Adrenal tumours	1					1		1
Thyroid tumours		1			1			
Lymphoma/leukaemia		1		2				
Pancreatic tumours							2	
Total	2	5	0	5	9	5	6	5

There was thus no evidence of greatly increased dust retention with increasing exposure at what by human standards are extremely high dose levels. From this it seems particularly unlikely that there would be an excessive build-up of lung dust because of "peak" doses considerably lower than those examined experimentally.

In this connection, however, it is important to consider just how different the factory situation is from the experimental design used in this study. In factories the overall dust levels are extremely low, usually well below 2 fibres per ml, so that a peak dose caused by a temporary machine defect probably lasting less than 1 h could easily be 100 times higher than this base-line and still result in a dust cloud of less than 1 mg/m^3 of air. A cloud of UICC chrysotile with a mass of 2 mg/m^3 has approximately 400 fibres per ml over 5 μm in length (Davis *et al.*, 1978). It was unfortunately not possible to compare the pathological effects of this extreme differential between "even" and "peak" doses in the laboratory. The dust load retained in a pair of rat lungs after a 12-month dusting period at 2 fibres/ml would have been too low to estimate reliably. It is also most unlikely that dusting at this level would have produced any detectable pathological changes in the lungs of rats over a $2\frac{1}{2}$–3-year life span. The equivalent "peak" dust cloud of 0·5 mg/m^3 of air for 1 h each week would result in an overall dose after 12 months of 140 times less than the 2mg dose used as a base-line for the peak experiments in the present study. This also would probably have been too low a dose to produce any detectable pathological change over the life span of a rat.

Regardless of the amount of dust retained in lung tissue following "peak" dosing, however, there did appear to be some differences in the levels of tissue damage resulting from this experimental procedure as compared to "even" dosing. Although there were no significant differences in tumour production in these experiments, the levels of early peribronchiolar fibrosis were significantly lower following "peak" inhalation of chrysotile and in the later stages of the experiment the levels of interstitial fibrosis found after "peak" dosing of both amosite and chrysotile were higher compared to the "evenly" dosed animals. In this case the differences were not statistically significant. It is possible that these tissue changes may indicate differences in the dust deposition patterns with "peak" or "even" dosing that do not show up in levels of total retained dust. It may be that short periods of high dose levels result in more dust remaining in the alveoli rather than being cleared towards the terminal bronchioles. There would thus be less tissue reaction near to the bronchioles and more in the lung parenchyma. This problem may be further elucidated by additional studies on long-term deposition and clearance of asbestos dust.

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